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## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number  
**WO 02/22156 A2**

(51) International Patent Classification<sup>7</sup>: **A61K 38/00**

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(21) International Application Number: PCT/US01/28886

(22) International Filing Date:  
17 September 2001 (17.09.2001)

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(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/662,994 15 September 2000 (15.09.2000) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:  
US 09/662,994 (CON)  
Filed on 15 September 2000 (15.09.2000)

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TOPICAL FORMULATIONS FOR DELIVERY OF INTERLEUKIN-11

(57) Abstract: Provided by the present invention are topical formulations of Interleukin-11 and methods for treating a variety of disorders, including inflammatory bowel diseases (e.g., Crohn's disease, ulcerative colitis, indeterminate colitis, and infectious colitis), mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis, and proctitis), necrotizing enterocolitis, inflammatory skin disorders (e.g., psoriasis, atopic dermatitis, and contact hypersensitivity), aphthous ulcers, pharyngitis, esophagitis, peptic ulcers, gingivitis, periodontitis, and ocular diseases (e.g., conjunctivitis, retinitis, and uveitis).

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## TOPICAL FORMULATIONS FOR DELIVERY OF INTERLEUKIN-11

### CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. Patent Application Serial No. 09/179,026  
5 filed October 26, 1998, which is a continuation-in-part of U.S. Patent Application Serial  
No. 08/892,407, filed July 15, 1997, now Patent No. 5,948,402, which is a divisional  
of U.S. Patent Application Serial No. 08/495,724, filed June 27, 1995, now Patent No.  
5,679,339, issued October 21, 1997.

### FIELD OF INVENTION

10 The present invention relates generally to novel compositions and methods for  
topical delivery of interleukin-11 (IL-11). In preferred embodiments, patients are treated  
employing topical delivery of recombinant human IL-11 for inflammatory bowel diseases  
(e.g., Crohn's disease, ulcerative colitis, indeterminate colitis, and infectious colitis),  
mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis, and proctitis),  
15 necrotizing enterocolitis, inflammatory skin disorders (e.g., psoriasis, atopic dermatitis,  
and contact hypersensitivity), aphthous ulcers, pharyngitis, esophagitis, peptic ulcers,  
gingivitis, periodontitis, and ocular diseases (e.g., conjunctivitis, retinitis, and uveitis).

### BACKGROUND OF THE INVENTION

Inflammatory responses include a broad range of host reaction to a variety of  
20 insults, such as injury, infection, or rejection. It is the overproduction of mediators that  
is believed to be associated with a broad range of disorders, including AIDS, arthritis  
(rheumatoid arthritis, osteoarthritis, spondyloarthropathies), antibiotic-induced diarrheal  
diseases, multiple sclerosis, osteoporosis, gingivitis, peptic ulcer disease, esophagitis,  
diabetes, retinitis, uveitis, reperfusion injury after myocardial infarction, cerebral  
25 vascular accident, aphthous ulcers (oral), atherosclerosis, tumor metastases, asthma,  
preeclampsia, pancreatitis, psoriasis, infertility and allergic disorders such as rhinitis,  
conjunctivitis, and urticaria.

Some of these disorders and their symptoms are briefly summarized below. According to the methods of the present invention, IL-11 is administered topically to modulate the host's over reaction at the site of insult, thereby treating the following disorders.

5           **Inflammatory Bowel Disease:**       Inflammatory bowel disease is the term generally applied to four diseases of the bowel, namely Crohn's disease, ulcerative colitis, indeterminate colitis, and infectious colitis.

**Ulcerative Colitis:**   Ulcerative colitis is a chronic inflammatory disease of unknown etiology afflicting the large intestine. The course of the disease may be  
10 continuous or relapsing, mild or severe. The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkühn. Coalescence of these distended and ruptured crypts tends to separate the overlying mucosa from its blood supply, leading to ulceration. Signs and symptoms of the disease include cramping, lower abdominal pain, rectal bleeding, and frequent, loose discharges  
15 consisting mainly of blood, pus, and mucus with scanty fecal particles. A total colectomy may be required for acute severe or chronic, unremitting ulcerative colitis.

**Crohn's Disease:**   Crohn's disease (also known as regional enteritis or ulcerative ileitis) is also a chronic inflammatory disease of unknown etiology but, unlike ulcerative colitis, it can affect any part of the bowel. The most prominent feature of the  
20 disease is the granular, reddish-purple edematous thickening of the bowel wall. With the development of inflammation, these granulomas often lose their circumscribed borders and integrate with the surrounding tissue. Diarrhea and obstruction of the bowel are the predominant clinical features. As with ulcerative colitis, the course of the disease may be continuous or relapsing, mild or severe but, unlike ulcerative colitis, it is not  
25 curable by resection of the involved segment of bowel. Most patients with Crohn's disease require surgery at some point, but subsequent relapse is common and continuous medical treatment is usual.

**Mucositis:**       Mucositis involves ulcerative breakdown of mucosal epithelial tissue, and is literally defined as inflammation of the mucous membrane. The  
30 pathophysiology of mucositis in response to toxic insults to the mucosa by chemotherapy

or by ionizing radiation is complex and involves a cascade of interactions among cells, cytokines and the oral microflora. The underlying premise for susceptibility of the mucosa of the oropharynx and gastrointestinal tract to chemotherapy or radiation damage is related to rapid epithelial stem cell turnover. Mucositis may be characterized by the

5 following phases:

1. Early inflammatory phase characterized by release of inflammatory cytokines in response to local tissue damage caused by cytotoxic agent(s);

2. Epithelial phase characterized by death of basal cells, which hinders re-population of the epithelium. This inability to regenerate leads to atrophy followed by  
10 ulceration. The ulceration represents loss of an important anatomic barrier at a site of local microflora;

3. Infection phase characterized by local invasion of microflora that results in an inflammatory response to the local infection. The inflammation results in additional local tissue damage and possibly erosive ulceration; and

15 4. Healing phase characterized by resolution of the infection and regeneration of epithelium.

Oral mucositis produces the following clinical symptoms and signs resulting from cellular damage: 1) sensation of dryness; 2) asymptomatic redness and erythema; 3) solitary white elevated desquamative patches which are painful upon pressure contact;  
20 and 4) large, painful, contiguous pseudomembranous lesions associated with dysphagia and decreased oral intake. These spontaneously painful lesions histopathologically show loss of epithelial cells to the basement membrane, which exposes the connective tissue stroma with its associated innervation.

As with oral mucosa, gastrointestinal mucosal damage results from disturbance  
25 of cellular mitosis that leads to reduction in the turnover rate of the basal cells of the intestinal crypts. The symptoms and signs of gastrointestinal mucositis include tenesmus (painful ineffectual straining at stool), pain, bleeding, diarrhea, telangiectasia (neovascularization), and progression to ulceration. Early signs of diarrhea include increased stool frequency, loose or watery stool, food aversion, increased bowel sounds,  
30 abdominal pain, and some loss of skin turgor indicative of dehydration. When the

diarrhea is severe it may be associated with mucosal ulceration, bleeding, intestinal perforation and proctitis. Stool exam may reveal occult blood and fecal leukocytes.

**Necrotizing Enterocolitis:** Necrotizing enterocolitis is an inflammatory disease of unknown etiology that afflicts between 1-5% of all infants admitted to neonatal intensive care units, most of whom are premature infants. Signs and symptoms include abdominal distention, gastrointestinal hemorrhage, and feeding intolerance. The disease most often involves the ileum and colon, and is characterized by loss of epithelium and submucosal edema, ulcerations, and, in severe cases, transmural necrosis.

**Psoriasis:** Psoriasis is a chronic inflammatory skin disorder involving hyperproliferation of the epidermis and inflammation of both the epidermis and the dermis. Macrophage, T-cells and neutrophil infiltration of the dermis and epidermis is seen, and proinflammatory mediators are released from these activated cells.

**Aphthous Ulcers (oral):** Although the cause of aphthous ulcers remain unknown, many physicians believe they are caused by autoimmune phenomena, which cause the destruction of discrete areas of the oral mucosa which leads to oral ulceration. Among the cytokines present in these active areas of ulceration, TNF- $\alpha$  appears to play a predominant role.

**Gingivitis:** Adult periodontitis is strongly associated with infection by *Porphyromonas gingivalis*. Proteolytic enzymes, which are produced in large quantity by this bacteria, are considered as important pathogenic agents. The increased production and flow of gingival crevicular fluid (GCF) is an important change in gingival tissues during periodontal infection, correlating with clinical indices of gingival inflammation. Indeed, salivary protein and albumin concentrations of individuals with periodontitis, which are an indication of plasma leakage due to vascular permeability enhancement (VPE), are significantly increased compared to healthy subjects. The production of GCF appears dependent on VPE induced at periodontitis sites, presumably involving proteinase(s) of *P. gingivalis* in their generation.

**Esophagitis:** The most common cause of esophagitis is the chronic reflux of hydrochloric acid from the stomach due to inefficiency of the cardiac sphincter of the stomach. The chronic presence of acid in the lower esophagus leads to damage of the

esophageal mucosa. In the most severe form, a syndrome called Barrett's esophagus can develop which often leads to esophageal cancer. Other causes of esophagitis include parenteral chemotherapy and ionizing radiation, associated with radiation therapy for cancer in the thoracic cavity.

- 5           **Retinitis:** Inflammation of the light sensitive retina, retinitis, can occur due to a variety of viral, bacterial or autoimmune etiologies. The end result is destruction of the retina and loss of sight.

- Uveitis:** Inflammation of the anterior portion of the eye its associated structures, the iris and cornea occurs with a relatively high frequency in patients with autoimmune  
10 disorders.

- Peptic Ulcer Disease:** Inhibition of gastric acid secretion with  $H_2$ -receptor antagonists and, more recently, blockers of  $H^+, K^+$ -ATPase (also known as the proton pump) has been the mainstay of therapy for peptic ulcer disease. The pathophysiology of peptic ulcers remains obscure. An appreciation of the complexity of the physiology  
15 of the gastric mucosa has led to a hypothesis that peptic ulcers are the result of an imbalance in the relative importance of aggressive (acid, pepsin) and protective (mucus, bicarbonate, blood flow, prostaglandins, *etc.*) factors. Infection of the mucosa of the human gastric antrum with the bacterium *Helicobacter pylori* has been widely accepted as the cause of chronic, active, type B gastritis. Further, this form of gastritis has been  
20 linked directly to peptic ulcer disease by studies showing that eradication of *H. pylori* reverses this gastritis and prevents duodenal ulcer relapse. Because cytokines are the principal mediators by which immune/inflammatory cells communicate with each other and with other cells, it is likely that these small peptides are involved in the pathogenesis of chronic active type B gastritis and the resulting peptic ulcer disease.

- 25           Some cytokines (IL-1, epidermal growth factor, transforming growth factor- $\alpha$ , acidic and basic fibroblast growth factors) tip the balance towards peptic ulcer healing; others (tumor necrosis factor- $\alpha$ ) appear to have no effect; still others (IL-4) may even cause gastrointestinal damage.

The infiltration and activation of multiple types of inflammatory cells result in a series of degenerative changes in the vasculature of the affected area, as well as inciting damage of the surrounding parenchymal tissue.

#### BRIEF SUMMARY OF THE INVENTION

5        Provided by the present invention are novel compositions for the topical administration of IL-11 in order to treat a number of disorders where such administration is preferable to systemic administration. Among the reasons that local administration may be preferred are the ease of administering a topical formulation compared to administration of subcutaneous injectable formulations. In certain classes of patients,  
10    the toxicity profile of chemotherapeutic agents may be such that concurrent parenteral administration of IL-11 is relatively unsuited. Other patients may have medical conditions for which the adverse event profile of parenteral IL-11 is relatively unsuited.

      According to the present invention, IL-11, analogs, and derivatives thereof, are administered to patients, either prophylactically or at the onset of symptoms associated  
15    with the aforementioned disorders, through one of several forms of local administration described below. IL-11 can be administered in suitable pharmaceutically acceptable carriers either alone or in combination with other conventional agents useful in alleviating the symptoms associated with the aforementioned disorders.

      In one embodiment, the present invention comprises preparations of IL-11 which  
20    are suitable for oral delivery to the mouth. Suitable oral preparations may be prepared with aqueous-based solutions such as sodium bicarbonate (e.g., Brioschi®), or in gels and suspensions for topical administration in the mouth. Oral preparations may also take the form of patches for delivery of IL-11 to the mouth via sustained release. Additional oral preparations may comprise IL-11 in the form of a lozenge or an uncoated tablet  
25    which is retained in the mouth. The oral preparations are particularly well-suited for disorders and inflammatory responses involving the mucosa of the head, neck and/or mouth. Such conditions include oral mucositis, pharyngitis, esophagitis, gingivitis, periodontitis, and aphthous ulcers (oral). Such conditions may result, for example, from

chemotherapy or radiotherapy for head and neck cancer, cervical esophageal cancer or lung cancer.

In other embodiments, the present invention comprises preparations of IL-11 which are suitable for topical delivery for mucosa and/or dermis. Such topical preparations may be prepared in the form of aqueous-based solutions, gels, ointments or creams for topical administration, as gels and suspensions for cervical administration, as pills, tablets, capsules or suppositories for immediate or sustained release to the gastrointestinal tract, or in the form of solution for enema. Such topical preparations are especially suited for treatment of disorders relating to local regions, such as psoriasis, as well as inflammatory bowel disease, esophagitis, and gastrointestinal mucositis. Some of these conditions may result, for example, from chemotherapy and/or radiotherapy for colorectal cancer, prostate cancer, cervical esophageal cancer or lung cancer.

Suitable doses of IL-11 are generally in the range of between about 1 and about 250  $\mu\text{g/kg}$  body weight, and may be administered from once a week up to about six times daily. Treatment may continue for a period of between one day and six months, or for as long as is deemed necessary and safe in the treatment of the aforementioned disorders, as is readily ascertained by standard tests by the attending physician, depending upon the nature of the disorder being treated.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is an graphic illustration of the Kaplan-Meier plot of percentage of survival in neutropenic animals treated with rhIL-11 administered topically in the intestine via orogastric feeding as described in detail in Example 6 below.

#### **DETAILED DESCRIPTION OF THE INVENTION**

Provided by the present invention are methods and compositions for topical administration of IL-11, for the treatment of various disorders, such as inflammatory bowel diseases (e.g., Crohn's disease, ulcerative colitis, indeterminate colitis, and infectious colitis), mucositis (e.g., oral mucositis, gastrointestinal mucositis, nasal mucositis, and proctitis), necrotizing enterocolitis, aphthous ulcers, psoriasis, pharyngitis, esophagitis, peptic ulcers, gingivitis, periodontitis, and ocular diseases (e.g., conjunctivitis, retinitis, and uveitis).

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred  
5 methods and materials are described. For purposes of the present invention, the following terms are defined below.

As used herein, the term "topical" refers to application at the site of inflammation (i.e., non-systemic administration), and includes, for example, oral, intranasal, intrabronchial, topical, and rectal routes of administration. The term "topical  
10 formulation" refers to a pharmaceutical formulation which is suitable for topical administration of the active ingredient.

As used herein, the term "pharmaceutically acceptable" refers to a carrier medium which does not interfere with the effectiveness of the biological activity of the active ingredient(s) and which is not toxic to the host to which it is administered.

15 As used herein, the terms "therapeutically effective amount" and "therapeutically effective dose" as applied to the active ingredient refers to the amount of the component in the composition or administered to the host that results in an increase in the therapeutic index of the host. The "therapeutic index" can be defined for purposes herein in terms of efficacy, i.e., extent of reduction or inhibition of inflammation. Suitable  
20 doses of the active ingredient can be determined using well-known methods, a variety of which are known and readily available in the pharmaceutical sciences, including, for example, measurement of markers associated with the disorder (e.g., TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and/or IL-12 p40 mRNA), the biological effects of TNF- $\alpha$ , and decreased symptomatology.

25 Interleukin 11 (IL-11) is a pleiotropic cytokine that stimulates primitive lymphohematopoietic progenitor cells and synergizes with other hematopoietic growth factors to stimulate the proliferation and maturation of megakaryocytes. IL-11 is described in detail in International Application PCT/US90/06803, published May 30, 1991, as well as in U.S. Patent No. 5,215,895; issued June 1, 1993. A cloned human  
30 IL-11 was previously deposited with the ATCC, 12301 Parklawn Drive, Rockville,

Maryland, on March 30, 1990 under ATCC No. 68284. Moreover, as described in U.S. Patent No. 5,270,181, issued December 14, 1993, and U.S. Patent No. 5,292,646, issued March 8, 1994, IL-11 may also be produced recombinantly as a fusion protein with another protein. IL-11 can be produced in a variety of host cells by resort to now  
5 conventional genetic engineering techniques. In addition, IL-11 can be obtained from various cell lines, for example, the human lung fibroblast cell line, MRC-5 (ATCC Accession No. CCL 171), and Paul *et al.*, the human trophoblastic cell line, TPA30-1 (ATCC Accession No. CRL 1583). A cDNA encoding human IL-11, as well as the deduced amino acid sequence (amino acids 1 to 199), is described in *Proc. Natl. Acad.*  
10 *Sci. USA* 87:7512 (1990). U.S. Patent No. 5,292,646, *supra*, describes a des-Pro form of IL-11 in which the N-terminal proline of the mature form of IL-11 (amino acids 22-199) has been removed (amino acids 23-199). As is appreciated by one skilled in the art, any form of IL-11 which retains IL-11 activity, such as variants through the substitution or deletion of amino acids, analogs and derivatives of IL-11, is useful  
15 according to the present invention. The disclosure of each of the above publications is hereby incorporated by reference for the contents thereof.

In addition to recombinant techniques, IL-11 may also be produced by known conventional chemical synthesis. Methods for constructing the polypeptides useful in the present invention by synthetic means are known to those of skill in the art. The  
20 synthetically constructed cytokine polypeptide sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with the natural cytokine polypeptides are anticipated to possess biological activities in common therewith. Such synthetically constructed cytokine polypeptide sequences or fragments thereof, which duplicate or partially duplicate the functionality thereof may also be used  
25 in the method of this invention. Thus, they may be employed as biologically active or immunological substitutes for the natural, purified cytokines useful in the present invention.

Modifications in the protein, peptide or DNA sequences of these cytokines or active fragments thereof may also produce proteins which may be employed in the  
30 methods of this invention. Such modified cytokines can be made by one skilled in the

art using known techniques. Modifications of interest in the cytokine sequences, *e.g.*, the IL-11 sequence, may include the replacement, insertion or deletion of one or more selected amino acid residues in the coding sequences. Mutagenic techniques for such replacement, insertion or deletion are well known to one skilled in the art. (See, *e.g.*,  
5 U. S. Patent No. 4,518,584.)

Other specific mutations of the sequences of the cytokine polypeptides which may be useful therapeutically as described herein may involve, *e.g.*, the insertion of one or more glycosylation sites. An asparagine-linked glycosylation recognition site can be inserted into the sequence by the deletion, substitution or addition of amino acids into  
10 the peptide sequence or nucleotides into the DNA sequence. Such changes may be made at any site of the molecule that is modified by addition of O-linked carbohydrate. Expression of such altered nucleotide or peptide sequences produces variants which may be glycosylated at those sites.

Additional analogs and derivatives of the sequence of the selected cytokine which  
15 would be expected to retain or prolong its activity in whole or in part, and which are expected to be useful in the present method, may also be easily made by one of skill in the art. One such modification may be the attachment of polyethylene glycol (PEG) onto existing lysine residues in the cytokine sequence or the insertion of one or more lysine residues or other amino acid residues that can react with PEG or PEG derivatives into  
20 the sequence by conventional techniques to enable the attachment of PEG moieties.

Additional analogs of these selected cytokines may also be characterized by allelic variations in the DNA sequences encoding them, or induced variations in the DNA sequences encoding them. It is anticipated that all analogs disclosed in the above-referenced publications, including those characterized by DNA sequences capable of  
25 hybridizing to the disclosed cytokine sequences under stringent hybridization conditions or non-stringent conditions (Sambrook *et al.*, Molecular Cloning. A Laboratory Manual, 2d edit., Cold Spring Harbor Laboratory, New York (1989)) will be similarly useful in this invention.

Also considered as derivatives useful in these methods are fusion molecules,  
30 prepared by fusing the sequence or a biologically active fragment of the sequence of one

cytokine to another cytokine or proteinaceous therapeutic agent, *e.g.*, IL-11 fused to IL-6 (see, *e.g.*, methods for fusion described in PCT/US91/06186 (WO92/04455), published March 19, 1992). Alternatively, combinations of the cytokines may be administered together according to the method.

5           Thus, where in the description of the methods of this invention IL-11 is mentioned by name, it is understood by those of skill in the art that IL-11 encompasses the protein produced by the sequences presently disclosed in the art, as well as proteins characterized by the modifications described above yet which retain substantially similar activity. Standard laboratory tests are utilized to monitor progress of the treatment.

10       Levels of TNF- $\alpha$  in serum or the biologic effects of TNF- $\alpha$  could be followed in a variety of these diseases. Decreased symptomatology could also be used to monitor the effectiveness of treatment as is well known to physicians skilled in the art of treating such disorders. Treatment is preferably prophylactic, but may also be at the onset of symptoms associated with the aforementioned disorders.

15           In one aspect of the invention, IL-11 is used in a therapeutic composition to alleviate or prevent the onset of symptoms associated with an inflammatory disorder. While it is possible to administer IL-11 alone, it is believed preferable to present it as part of a pharmaceutical formulation. In accordance with this aspect of the invention, the pharmaceutical compositions comprise IL-11 in a therapeutically effective dose

20       together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. A wide variety of pharmaceutically acceptable carriers are known to those of skill in the art. See, *e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 17th ed. (1985), which is incorporated by reference herein. Preferred carriers include inert, non-toxic solids (*e.g.*, dextrose, dextrin,

25       cellulose, pectin, starch, lactose, sucrose, and calcium phosphate, ), semi-solids (*e.g.*, glycerol stearate, polyethylene glycol, stearic acid, agar, gelatin, and propylene glycol) and liquids (*e.g.*, buffered saline, water, an organic solvent, and pharmaceutically acceptable oils or fats).

          As will be appreciated by those of skill in the art, the preferred form of the

30       pharmaceutical composition of IL-11 will depend on the intended mode of

administration, which in turn will depend on the location and nature of the inflammatory disorder to be treated. For example, delivery to the mouth (e.g., for treatment of oral mucositis, aphthous ulcers, gingivitis, periodontitis), head and/or neck (e.g., for treatment of pharyngitis, esophagitis) can be in the form of aqueous-based oral solutions, suspensions, emulsions, syrups, elixirs, gels, patches, lozenges, tablets, or capsules. Delivery to the gastrointestinal tract (e.g., for treatment of gastrointestinal mucositis, peptic ulcers and inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, indeterminate colitis, and infectious colitis) can be in the form of oral solutions, gels, suspensions, tablets, capsules, and the like. IL-11, either alone or in combination with other components in a pharmaceutical composition, can be added to infant formula for delivery to the gastrointestinal tract of an infant suffering from or susceptible to necrotizing enterocolitis. It is also possible to formulate the IL-11 preparation for rectal administration (e.g., for treatment of proctitis), e.g., in the form of enema, suppositories, rectal-foam, and the like. Delivery to the eye (e.g., for treatment of ocular diseases such as conjunctivitis, retinitis, and uveitis) can be in the form of solutions, gels, or suspensions. Delivery to the nose (e.g., for treatment of nasal mucositis) can be in the form of solutions, gels, or suspensions. The intranasal formulations may be formulated, for example, into an aqueous or partially aqueous solution, which can then be utilized in the form of a nasal drop or an aerosol. Delivery to the skin (e.g., for treatment of psoriasis) can be in the form of aqueous-based solutions, gels, suspensions, lotions, creams, ointments, patches, and the like.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral administration include water (partially containing additives as above), alcohols (including monohydric alcohols and polyhydric alcohols, e.g., glycols)

and their derivatives, oils (e.g., peanut oil, sesame oil, olive oil, and coconut oil), and combinations of the above. Compositions comprising such carriers and adjuvants may be formulated using well known conventional materials and methods. Such materials and methods are described, for example, in *Remington's Pharmaceutical Sciences*,  
5 *supra*. Infant formula is also a suitable liquid carrier, particularly when the pharmaceutical composition is used to treat or prevent necrotizing enterocolitis.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating  
10 agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablet preferably contain up to 99% of the active ingredient, and may be formulated for  
15 immediate and/or sustained release of the active ingredient. Suitable solid carriers include, for example, calcium or sodium phosphate, magnesium stearate, talc, sugars, glycine, lactose, dextrin, starch, gelatin, cellulose, cellulose derivatives (e.g., methyl cellulose, hydroxypropylmethyl cellulose, and sodium carboxymethyl cellulose), polyvinylpyrrolidone, low melting point waxes, and combinations of the above.

20 Oral tablets may be prepared using a variety of well known methods and in a variety of conventional forms. Exemplary forms include dry powder compaction tablets, micro-particulate systems (e.g., wherein the active ingredient is spray-dried onto a scaffold particle), and hard or soft-gel capsules. The tablets may be optionally covered with an enteric coating, which remains intact in the stomach, but will dissolve and  
25 release the contents of the tablet once it reaches the small intestine. Most currently used enteric coatings are those which remain undissociated in the low pH environment of the stomach, but readily solubilize when the pH rises to about 4 or 5. A number of commercially available enteric coatings may be used depending on the target part of the intestinal tract, i.e., the site of the inflammatory bowel disorder in the patient. Such  
30 coatings include, for example, methacrylic acid-methacrylic acid ester-based copolymer,

which is sold under the trade name "Eudragit"; anionic water-soluble, polymer cellulose ether, which is sold under the trade name "Aqualon"; cellulose acetate phthalate; polyvinyl acetate phthalate; hydroxypropyl methylcellulose phthalate; and the like. Compositions comprising such carriers and adjuvants may be formulated, and tablets  
5 prepared from such compositions, using well known conventional materials and methods. Such materials and methods are described, for example, in *Remington's Pharmaceutical Sciences, supra*.

In one embodiment of the invention, the pharmaceutical composition comprises one or more sustained or controlled release excipients such that a slow or sustained,  
10 preferably constant, release of the active ingredient is achieved. A wide variety of suitable excipients are known in the art and are not a part of this invention. Such sustained/controlled release excipients and systems are described, for example, in U.S. Patent No. 5,612,053 (Baichwal et al.), U.S. Patent No. 5,554,387 (Baichwal), U.S. Patent No. 5,512,297 (Baichwal), U.S. Patent No. 5,478,574 (Baichwal et al.), and  
15 U.S. Patent No. 5,472,711 (Baichwal et al.), each of which is incorporated by reference herein. If desired, the pharmaceutical composition can be formulated to provide a pulse dose of the active ingredient. A variety of pulse-dose systems, which provide low or high-pulsed doses, are known in the art and are not a part of this invention. Such pulse-dose formulations are described, for example, in Y.H. Bae, "Stimuli-Sensitive Drug  
20 Delivery," *Controlled Drug Delivery* (ed. K. Park) ACS Press, Washington, D.C. (1997); *Drugs* (1982) 23:207-226; *Fertil.Steril.* (1983) 39:695-699, 1983; *Brit. J. Cancer* (1982) 45:86-94; *Int. J. Radiat. Oncol. Biol. Phys.* (1982) 8:915-919; *J. Clin. Endocrinol. Metab.* (1981) 53:184-91; and *Diabetes* (1977) 26:571-581.

In another embodiment of the invention, the pharmaceutical composition is  
25 formulated to provide direct and/or targeted delivery of the active ingredient to a specific anatomic site or sites within the gastrointestinal tract; e.g., the duodenum, jejunum, ileum, cecum and/or colon, each of which is believed to contain receptors for IL-11. Methods for providing targeted delivery of macromolecules, including proteins and polypeptides, to specific tissues or organs within a mammalian host are well known in  
30 the art and not a part of this invention. Such targeted delivery systems are described,

for example, in R.J. Mersny, "Site-Specific Drug Delivery in the Gastrointestinal Tract," *Controlled Drug Delivery, supra*; M. Mezei and D. Meisner, "Liposomes and Nanoparticles as Ocular Drug Delivery Systems," *Biopharmaceutics of Ocular Drug Delivery*, Ch. 6 (ed. P. Edman) CRC Press, Boca Raton, FL (1992); T.L. Bowersock and H. Hogenesch, "Oral Immunization Using Microparticles," *Controlled Drug Delivery, supra*; M. Mezei, "Liposomes in Topical Application of Drugs," *Liposomes as Drug Carriers: Trends and Progress* (ed. G. Gregoriadis), J. Wiley & Sons Publ. USA (1988), pp. 663-677; and J.J. Berti and J.J. Lipsky, "Transcutaneous Drug Delivery: A Practical Review," *Mayo CLW Proc.* (1995) 70:581-586.

10 IL-11, either alone or in combination with other therapeutic agents, may also be administered topically in the form of a dermal patch or transdermal delivery system. Such patches and systems are especially suited for treatment of inflammatory skin disorders such as psoriasis, and for inflammatory disorders involving the mucosa of the head, neck and/or mouth, e.g., oral mucositis, pharyngitis, esophagitis, gingivitis, 15 periodontitis, and aphthous ulcers. In this embodiment of the invention, the pharmaceutical composition may be administered through the use of a dermal patch containing the active ingredient(s) and a carrier that is inert to the active ingredient(s), non-toxic to the skin or mucosal epithelium, and allows delivery of the agent to the dermis and/or epithelium. Dermal patches and delivery systems, utilizing active or 20 passive transdermal delivery carriers, comprising IL-11 may be prepared using well known methods and materials, including, for example, microporous membranes, silicon polymers and diffusion matrixes. Such materials and methods are described, for example, in *Remington's Pharmaceutical Sciences, supra*.

In yet another embodiment of the invention, at least one proteinase inhibitor is 25 combined with IL-11 in a therapeutic composition to treat inflammatory diseases. In accordance with this aspect of the invention, the pharmaceutical composition comprises IL-11 and one or more proteinase inhibitors, which stabilize the IL-11 molecule against proteolytic degradation. It may be preferable to include a proteinase inhibitor in the pharmaceutical composition to protect IL-11 against proteolytic enzymes in the 30 gastrointestinal tract, particularly in rectal formulations. A wide variety of suitable

proteinase inhibitors are known in the art and are not a part of this invention. Suitable proteinase inhibitors include, for example, aprotinin, a kallikrein-trypsin inhibitor, and protein serine proteinase inhibitors such as  $\alpha$ -macroglobulin, soybean trypsin inhibitor, and ovomucoid.

5           A suitable treatment regimen for patients undergoing treatment, including for example prophylactic treatment, may be determined by the attending physician based upon such factors as the patient's age, sex, weight, and general health. Generally, a suitable dose of IL-11 ranges broadly, preferably between about 1 and about 250 microgram ( $\mu$ g) per kilogram (kg) of body weight of recipient per treatment. Another  
10       suitable dose may be in the range of about 1 to about 100  $\mu$ g per kg of body weight, and more preferably in the range of about 10 to about 50  $\mu$ g per kg of body weight. If desired, these doses can be adjusted to units. A unit is conventionally described as the concentration of polypeptide which leads to half-maximal stimulation in a suitable assay, *e.g.*, for IL-11, the T1165 assay described in PCT/US90/06803. Doses may be  
15       administered daily for between one day and six months, or for as long as is deemed necessary and safe, as is readily ascertained by standard tests by the attending physician, depending upon the nature of the disorder being treated. Where appropriate, the dosages may be adjusted upward or downward, for example, a dosing regimen requiring administration of IL-11 at a dose of 25  $\mu$ g/kg, daily for one week, or fewer days, or  
20       multiple weeks if indicated. The progress of treatment is appropriately monitored by measurement of markers associated with the disorder being treated to determine if such a dose results in a decrease of for example, TNF- $\alpha$  levels (or corresponding marker) and if not, increasing the dose two-fold for an additional time period of treatment and measurement of marker levels until an effective dosing regimen is reached.

25           The following examples illustrate the methods of the present invention and in particular the use of topical IL-11 formulations in treating Crohn's disease, chemotherapy-induced ulcerative mucositis, colitis (colonic ulcers), psoriasis, and inflammatory bowel disease. However, as the skilled artisan will recognize from the present disclosure, numerous modifications and variations are possible and comprised

within the present invention. The examples are not exhaustive, and do not limit the scope of the invention in any way.

#### **EXAMPLE 1: TREATMENT OF INFLAMMATORY BOWEL DISEASE**

In two well-established models it has been shown that subcutaneous rhIL-11 is able  
5 to significantly reduce the clinical signs and histologic lesions of gastrointestinal inflammation: acetic acid-induced colitis, a model of acute injury; and the HLA-B27 transgenic rat, a model of chronic inflammation. While conducting further studies of the mechanism of rhIL-11 action, oral topical activity was seen in hamsters with 5-FU induced oral mucositis and mucosal activity was documented in *in vitro/ ex vivo* studies of  
10 intestinal transport. Subsequently, these models have been used to confirm the effectiveness of topically and orally administered rhIL-11.

Applicants first examined local topical delivery of rhIL-11 to the colon by enema. To determine the stability of rhIL-11 in the lumen of the gut, rhIL-11 and intestinal chyme from HLA-B27 rats were combined and incubated at 37 °C for up to several hours  
15 in the presence or absence of a proteinase inhibitors. Fluid samples were analyzed periodically by SDS-PAGE. The addition of a proteinase inhibitor, avian ovomucoid, stabilized rhIL-11 against the proteolytic degradation observed in the rat chyme. Next, twenty-three HLA-B27 rats (22-23 weeks old) were randomized to one of six treatment groups: rhIL-11 Vehicle; 50µg/ml ovomucoid; rhIL-11 200 µg/ml; or rhIL-11 at 50, 200  
20 or 800 µg/ml combined with 50µg/ml of ovomucoid. Animals received a colonic irrigation (1ml) of the respective formulations, Monday, Wednesday and Friday, for three weeks. Stool character was assessed daily. At the end of the experiment, terminal blood samples were obtained, and then the animals were euthanized by an overdose of anesthetic. Platelet counts and colonic mucosal lesions were quantitated and analyzed as described  
25 previously. Platelet counts were not different in any of the groups. In the vehicle treated animals, normal, soft or diarrhea stools were present at 16.5, 33, or 50% of the total animal observation days, respectively. In the 50µg/ml+50µg/ml ovomucoid animals, normal, soft, or diarrhea stools were present at 65.9, 25, or 9.1% of the total animal observation days, respectively. Histologic lesion scores were 7.25±1.4 and 7.8±1.4 in the

vehicle and ovoinhibitor groups, respectively. ANOVA testing showed that all of the rhIL-11 lesion scores were lower than either vehicle or ovoinhibitor ( $P < 0.05$ ), and the lowest lesion score was in the 50 $\mu$ g rhIL-11 + 50 $\mu$ g ovoinhibitor group,  $2.1 \pm 0.4$ . Topically administered rhIL-11 was beneficial in the model of chronic inflammatory  
 5 bowel disease when combined with a proteinase inhibitor. Since this study was based around a dose of 200 $\mu$ g/ml, no dosing group of 50 $\mu$ g/ml without the proteinase inhibitor was included.

Next, the effects of lower doses of recombinant human interleukin eleven (rhIL-11) with or without Aprotinin (an FDA approved and marketed Proteinase inhibitor) delivered  
 10 by enema were assessed in the acetic acid model of acute colitis. Colitis was induced in the descending colons of 80 anesthetized Sprague-Dawley rats by intraluminal instillation of 10% acetic acid for 10 seconds on Day 0 of the study. The rats were randomly assigned to the 8 groups according to their different treatments as described below:

TABLE 1				
15	Group	No. Animals	Dosage	Schedule
	1 Vehicle	10	1ml.Buffer	3 days/wk/2wks.
	2 Aprotinin	10		50 $\mu$ g/ml 3
	days/wk/2wks.			
	3 & 4 rhIL-11	20		10 $\mu$ g/ $\mu$ g/ml 3days/wk/2wks
20	$\pm$ Aprotinin			
	5 & 6 rhIL-11	20		25 $\mu$ g/g/ml 3 days/wk/2wks
	$\pm$ Aprotinin			
	7 & 8 rhIL-11	20	50 $\mu$ g/ml	3 days/wk/2wks
	$\pm$ Aprotinin			

25 Peripheral hematologic parameters were assessed. On day 15, the animals were euthanized, and colons were removed for determination of lesion scores by gross examination and histologic evaluation. Platelet counts in all groups were in normal

range. All animals treated with acetic acid developed diffuse colonic lesions, characterized by ulceration, hemorrhage, edema, depletion of goblet cells, and infiltration of leukocytes. The gross and histologic lesions of all rhIL-11 treated animals were significantly reduced compared with vehicle controls on day 15. Administration of rhIL-11 10 $\mu$ g with 50 $\mu$ g Aprotinin 3 days/week/for 2 weeks or 50 $\mu$ g/ml rhIL-11 3 days/wk/2wks appeared to be most effective in this model of colonic mucosal injury.

#### **EXAMPLE 2: TREATMENT OF ORAL MUCOSITIS**

95 male Golden Syrian hamsters were equally divided into five groups. Group 1 [control] received phosphate buffered saline and 0.5% hamster serum (vehicle) topically on days 3-14. Groups 2 and 4 received 50  $\mu$ g IL-11 subcutaneously, twice daily, on days 0-14 and 3-14, respectively. Groups 3 and 5 received 100  $\mu$ g IL-11 topically, four times daily, on days 3-14 and 0-14, respectively. Ulcerative mucositis was induced by administration of 60 mg/kg body weight of 5-fluorouracil on days 0 and 2. The left buccal pouch was superficially irritated on day 4. Ulcerative mucositis was evaluated blindly starting on day 6 by scoring standardized photographs. The scoring was done on a scale of 0-10, with 10 being the most severe. Animals were weighed daily. Blood was taken from 3 animals per group on days 6, 10 and 14.

For the entire experiment, average mean ulcerative mucositis scores for all groups were significantly [ $p < 0.05$ ] lower than control. [G1=5.0; G2=3.8; G3=4.1; G4=3.3; G5=4.0]. Day 14 survival rates for G1 through G5 were 23%; 69%, 38%, 100% and 46%, respectively. Thus, topical administration of IL-11 was shown to favorably modify the course of chemotherapy-induced ulcerative mucositis relative to control.

The present investigation demonstrates the beneficial effects of a topical formulation of recombinant human IL-11 (rhIL-11) on the induction, course and resolution of chemotherapy-induced mucositis.

#### **Materials and Methods**

Male LVG Golden Syrian hamsters (Charles River Laboratories, Wilmington, MA), aged 5-6 weeks, were caged in small groups and fed standard hamster chow and

water *ad libitum*. All procedures were conducted in accordance with guidelines set by the Harvard Medical Area Standing Committee on Animals.

#### **Mucositis induction and evaluation**

All procedures were performed using anesthesia induced by diethyl ether. To induce mucositis, 5-fluorouracil was administered (60 mg/kg) by intraperitoneal injection on days 0 and 4. To mimic the functional trauma, which occurs in humans, the left buccal pouch mucosa was superficially irritated on day 4. Animals were weighed and observed daily through day 14. Mucositis was evaluated on days 6 to 14. To evaluate mucositis, animals were anesthetized and the cheek pouch was everted, immobilized, and photographed using a standard, validated technique. At the conclusion of the study, all film was developed simultaneously. Photographs were numbered, randomized and graded blindly by three independent observers using a 10-point scale in which mild mucositis equaled a score of 1 to 3, moderate 4 to 6, and severe greater than 6. Mild mucositis was defined as mucosal erythema with vasodilation. Mold mucositis was defined as ulceration with pseudomembrane formation, erythema and vasodilation, and severe mucositis consisted of diffuse ulceration with extensive areas of necrosis, pseudomembranes, erythema and vasodilation.

#### **Test groups and protocol**

Ninety-five animals were prospectively randomized into five equal groups. Each animal was individually numbered using an ear punch technique. The following groups were studied:

- |         |   |
|---------|---|
| Group 1 | Vehicle control, topical, four times daily, days 3 to 14 (day 0 = day of first chemotherapy injection). |
| Group 2 | 100 $\mu$ g IL-11 per day in a divided dose given twice daily, subcutaneously, days 0-14.               |
| Group 3 | 400 $\mu$ g IL-11 per day in a divided dose given four times daily, topically, days 3-14.               |
| Group 4 | 100 $\mu$ g IL-11 per day in a divided dose given twice daily, subcutaneously, days 3-14                |

Group 5      400  $\mu$ g IL-11 per day in a divided dose given four times daily, topically, days 0-14.

Animals in the Group 1 were treated with topical application of PBS containing 0.5% hamster serum. Animals in the test groups received rhIL-11 made up in PBS containing 0.5% hamster serum. Animals receiving topical therapy received treatment at 9 a.m., 11:30 a.m., 2 p.m. and 4:30 p.m. Animals receiving injections of IL-11 were treated at 9 a.m. and 4 p.m. Whole blood was obtained on days 6 and 14 to evaluate the number of platelets.

### Results

Cumulative mean mucositis scores for group 2 (3.0) and group 4 (2.7) were markedly less than that noted for the control (4.7). Topical application of rhIL-11 was of benefit (group 3 = 3.3; group 5 = 3.4), though slightly less efficacious than rhIL-11 delivered by the subcutaneous route. Interestingly, the efficacy of rhIL-11 given by either route was essentially comparable after day 10. Marked rhIL-11 benefit was noted from day 6 through day 14 among animals treated via the subcutaneous route. On days 6, 7, and 8, animals in group 2 (IL-11 from day 0) had slightly less mucositis than did animals in group 4 (IL-11 beginning on day 3). On day 9 this trend was reversed. No difference was seen for the remaining days of the study.

Schedule of rhIL-11 administration had no observed effect on its topical efficacy, with no significant differences observed between scores of animals in groups 3 and 5. Less mucositis was observed in animals treated with topically applied rhIL-11 throughout the course of the experiment. Reduction in mucositis scores was essentially equivalent between both topical groups. Interestingly, the effect of topically applied rhIL-11 was the same as the subcutaneously administered peptide from day 11 to the conclusion of the experiment.

Evaluation of the effect of rhIL-11 on moderate to severe mucositis demonstrated a similar trend. From day 6 to day 9, the percentage of control animals (group 1) with moderate to severe mucositis was markedly greater than that noted in animals treated with rhIL-11. In general, animals treated subcutaneously responded more favorably.

For example, on day 8, 67% of control animals had mucositis scores equal to or greater than 5 compared to 13% of animals in group 2 and 31% of animals in group 4. The frequency of animals in the topically treated groups was 42% (group 3) and 39% (group 5). This trend appears to have shifted on days 10 to 11 where it appears that topically treated animals did better than those injected with rhIL-11. While this is true of surviving animals, evaluation of survival data is of importance in interpreting this apparent trend.

The above results demonstrate that topical administration of rhIL-11 is beneficial *in vivo* for the treatment of oral mucositis.

### 10 EXAMPLE 3: TREATMENT OF COLITIS (COLONIC ULCERS)

The effects of three different dosages of recombinant human interleukin-11 (rhIL-11), given subcutaneously (SC) either prior to or subsequent to intracolonic administration of trinitrobenzene sulfonic acid (TNB), were studied in Sprague-Dawley rats. The TNB or control were given in a 40% ethanol solution to 312 anesthetized adult male rats allotted to one of 26 groups (n=12). Control groups were: subcutaneous (SC); saline alone; intrarectal (IR); 40% ethanol alone; TNB alone; 40% ethanol alone, and SC, rhIL-11 at the highest dosage alone and groups combining TNB with rhIL-11 therapy, testing three dosages (100, 300, and 1,000  $\mu\text{g/kg}$ ), given either before or after induction of colitis with TNB. Body weight changes were monitored. Rats were euthanized at 3 days, 7 days, or 14 days after TNB administration. At necropsy, samples were collected to evaluate fecal occult blood, mucosal myeloperoxidase activity and mucosal gross indexes of ulceration. Histopathologic and ultrastructural analyses of the colonic mucosa were performed. The TNB alone elicited a prolonged, severe colitis in treated animals, and the ethanol control group showed a short-lasting, less severe colonic inflammatory response. Colonic ulcer indexes of rhIL-11 treated rats showed a consistent, dose-related reduction in the severity of the TNB-induced colitis, whether the interleukin was given before or after the TNB. This reduction was significant ( $P < 0.05$ ) after administration of the intermediate (300  $\mu\text{g/kg}$ ) and highest (1,000  $\mu\text{g/kg}$ ) dose levels of rhIL-11, in the groups given rhIL-11 for 7 days after TNB.

Myeloperoxidase activity was increased during the TNB-induced colitis and was reduced by rhIL-11 administration ( $P < 0.01$ ). Fecal occult blood loss increased with colitis and paralleled its severity. rhIL-11 enhanced mucus production and decreased the severity of TNB-induced colitis.

#### 5    **EXAMPLE 4:            TREATMENT OF MUCOSITIS**

One of the dose-limiting toxicities of radiation therapy for head and neck cancer is the occurrence of oral mucositis. The effects of topical administration of IL-11 on this effect are examined in conjunction with at least one of several regimens employed for the treatment of advanced head and neck cancer. The specifics of the regimens vary, and may include external beam standard fractionated irradiation, hyperfractionated irradiation, or accelerated fractionated irradiation. Various chemotherapy agents may also be employed either sequentially or concurrently with radiotherapy. Chemotherapy agents frequently employed in this setting include 5-fluorouracil (5-FU), cisplatin, carboplatin, hydroxyurea, paclitaxel, and topotecan, either alone or in various combinations.

One regimen is outlined for the purpose of illustration. The regimen involves hyperfractionated external beam irradiation and concurrent chemotherapy with infusional 5-FU and cisplatin. This regimen has been employed for several years for patients with locally advanced squamous cell carcinoma of the head and neck. Patients receive 125 centi-Gray (cG) twice per week of external beam irradiation 5 days per week. The total planned dose is 7000 cG. During weeks 1 and 6 of radiotherapy, patients are treated with 5-FU and cisplatin. 5-FU is administered as a 5-day continuous infusion of 600 mg/m/day while cisplatin is administered as a bolus injection of 12 mg/m/day for 5 days. There is a 7-day scheduled treatment break after 4000 cG to allow mucositis to subside. The entire planned time for completion of the course of irradiation and concurrent chemotherapy is 7 weeks.

Patients are randomly assigned in a ratio of 2:1 to receive either topical IL-11 or placebo. Patients receiving IL-11 will receive either 50 ug/L or 150  $\mu$ g /mL in 15 to 30 mL of Brioschi (sodium bicarbonate). A 5 mg vial of IL-11 or excipient alone [placebo]

first will be resuspended with 1 mL water for injection (WFI). When 30 mL of Brioschi is used, 300  $\mu$ L (for 50 ug/mL dose) or 950  $\mu$ L (for the 150 ug/mL dose) containing either IL-11 or excipient alone will be removed from the vial and added to 30 mL Brioschi. This final preparation, containing either IL-11 or placebo excipient alone, is  
5 swished and swallowed twice daily for 6 to 8 weeks beginning the day before radiotherapy. All patients are assessed to determine the effects of the topical preparation of IL-11 on patients receiving chemotherapy and/or radiotherapy for head and neck cancer. Among the endpoints summarized are:

- Incidence of oral mucositis of Grade 3 or higher, as measured by the modified  
10 National Cancer Institute Common Toxicity Criteria;
- Duration of oral mucositis of Grade 3 or higher. Duration is measured as (1) the time from onset of Grade 3 or higher until resolution to less than Grade 3 and (2) the number of days of oral mucositis of Grade 3 or higher;
- Total duration of oral mucositis of any grade;
- 15 • Maximum severity of oral mucositis experienced during the study;
- Incidence of hospitalization due to oral mucositis; and
- Incidence of IV fluid resuscitation due to dehydration secondary to poor oral intake [secondary to mucositis].

Patients receiving IL-11 exhibit significant improvement in one or more of the above  
20 criteria.

#### **EXAMPLE 5: TREATMENT OF PSORIASIS**

The effects of IL-11 in modulating keratinocyte (the target cell type in a number of cutaneous inflammatory skin diseases, including psoriasis) growth and gene expression were assessed in vitro. When rhIL-11 (recombinant human IL-11) was added  
25 to a basal culture medium at a concentration of 100 ng/mL, it stimulated keratinocyte proliferation. RT-PCR analysis showed that keratinocytes grown in basal as well as enriched medium expressed IL-11 receptor  $\alpha$ -chain mRNA (IL-11R $\alpha$ ). rhIL-11 was able to stimulate phosphorylation of STAT-3 in keratinocytes when grown in basal medium. To assess whether rhIL-11 could affect the levels of surface intercellular adhesion

molecule-1 (ICAM-1) induced by IFN- $\gamma$  and TNF- $\alpha$ , keratinocytes were exposed to IFN- $\gamma$  and TNF- $\alpha$  for one hour before adding rhIL-11. Addition of rhIL-11 resulted in a significant decrease in the amount of surface ICAM-1. These results show that IL-11 has direct effects on keratinocytes, thereby downregulating cutaneous immune responses.

5    **EXAMPLE 6:            ADMINISTRATION OF IL-11 IN MULTIPARTICULATE  
                                 ORAL FORMULATION IN RATS**

                                 Multiparticulate formulations of rhIL-11, in which rhIL-11 was sprayed onto a sucrose bead and covered with a sealant and an enteric coating were prepared (1 mg active ingredient/100mg multiparticulates) and tested in the neutropenic rat model. This  
10    experimental model is designed to mimic the pathophysiological events that may occur in febrile neutropenic cancer patients. See, Opal, "Recombinant Human Interleukin-11 in Experimental Pseudomonas aeruginosa Sepsis in Immunocompromised Animals", *Journal Inf. Dis.* 178:1205-8 (1998) and Opal, "Additive Effects of Human Recombinant Interleukin-11 and Granulocyte Colony-Stimulating Factor in Experimental Gram-  
15    Negative Sepsis", *Blood* 93:3467-72 (1999). In the experimental model, neutropenic sepsis was caused by infecting 16 Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) with a virulent, scrum-resistant strain of Pseudomonas aeruginosa (strain 12.4.4, taken from a human blood isolate, Fisher-Devlin-Gnabasik Immuntotype VI, from a patient who died of sepsis), which generates an endogenously-  
20    mediated bacteremic infection. Cefamandole (Eli Lilly, Indianapolis, IN) was given at 100 mg/kg intramuscularly at days -4, -2, -1, 1, 3, 5, 7 and 9 to disrupt colonization resistance. Animals are rendered neutropenic through the intraperitoneal administration of cyclophosphamide (Sigma, St. Louis, MO) at time 0 (150 mg/kg) and again at day 3 (50 mg/kg) to induce a profound neutropenia (absolute neutrophil count  $< 50/\text{mm}^3$ ) for  
25    5-7 days. Body temperature was monitored with a digital noncontact infrared thermometer (Horiba; Markson Science, Phoenix, AZ). Beginning one day before beginning chemotherapy and bacterial challenge (i.e., at day -1), each rat was given one dose per day for 12 days of the microparticulate form of rhIL-11 (0.5 mg/kg) in sodium citrate buffer at pH 5 by orogastric feeding. Control rats (n=16) were given uncoated,

multiparticulate sucrose beads in sodium citrate buffer at pH 5 by orogastric feeding. Blood was drawn at days -4, 5 and 6 and tested for levels of circulating endotoxin.

Animals were examined daily, and necropsy examinations were done with wet-to-dry measurements of lung tissue and quantitative bacterial counts of lung tissue and blood using *Pseudomonas* isolation agar (Difco, Detroit, MI). Mucosal mass of 10-cm segments of jejunum were determined by the methods described in Fox, "Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis", *J Parenter Enteral Nutr* 12: 325-31 (1988). The terminal ileum, cecum, and proximal large bowel were isolated, instilled with formalin, preserved, and sectioned. Histopathologic evidence of mucosal injury was assessed by a pathologist who was blinded to the treatment assignment of each animal. A pathologic score of 0-4 (Normal, 0; mild, 1; moderate, 2; severe focal, 3; severe diffuse, 4) was used to quantify the degree of mucosal thinning and necrosis. Numeric data were analyzed by the Kruskal-Wallis one-way analysis of variance test. A Kaplan-Meier plot of percentage of survival was used to analyze outcome in each treatment group, and differences in survival time were measured by the Kruskal-Wallis test.  $P < 0.05$  was considered significant. See Figure 1.

Animals randomized to the control group developed a uniformly fatal bacteremic infection with *R. aeruginosa*, with multisystem infection and multiorgan failure. By day 12, all of the control rats had died. Administration of rhIL-11 resulted in a significantly improved survival (over 80%;  $P < 0.0001$ ). See Figure 1. Median survival time of rats given rhIL-11 was 10.2 days whereas the median survival time of rats given the control was 7.9 days ( $p < 0.01$ ). Mucosal mass of small intestine was maintained at near normal levels in the rhIL-11 treated rats (median = 138 mg/10 cm) and significantly reduced in the control (median = 75 mg/10 cm). Endotoxin levels were measured by a quantitative turbidimetric limulus amoebocyte lysate assay (Associates of Cape Cod, Woods Hole, MA). Levels of circulating endotoxin were significantly reduced in the rhIL-11 treated rats (0.4 ng/ml) compared to the control treated rats (4.04 ng/ml) and the quantitative microbiology for *P. aeruginosa* in liver and spleen tissue was significantly reduced in the rhIL-11 treated rats ( $\log_{10}$  0.1 CFU/gm) compared to the control rats ( $\log_{10}$  4.14 CFU/gm).

These finding indicate that orally administered rhIL-11 provides mucosal protection against chemotherapy-induced mucosal injury and maintains gut barrier function in experimental animal models. Oral rhIL-11 limits weight loss, preserves enterocyte mass, limits gut mucosal damage, decreases microbial invasion from the alimentary tract and endotoxin release from the gastro-intestinal mucosa and improved survival during the course of chemotherapy-induced neutropenia in experimental animals. This provides a new clinical strategy to gastro-intestinal protection during the course of chemotherapy-induced neutropenia.

While the present invention has been described in terms of specific methods and compositions, it is understood that variations and modifications will occur to those skilled in the art upon consideration of the present invention.

Numerous modifications and variations in the invention as described in the above illustrative examples are expected to occur to those skilled in the art and, consequently, only such limitations as appear in the appended claims should be placed thereon. Accordingly, it is intended in the appended claims to cover all such equivalent variations which come within the scope of the invention as claimed.

The disclosure of all of the publications cited herein are hereby incorporated by reference.

## WE CLAIM:

1. A method of treating Inflammatory Bowel Disease comprising administering to a patient a topical formulation of a pharmaceutical composition composed of a pharmaceutically effective amount of IL-11 and a carrier for delivery of IL-11 to the gastrointestinal tract.
2. The method of claim 1, wherein said carrier is an immediate release carrier.
3. The method of claim 2, wherein said immediate release carrier comprises a collection of multi-particulate particles.
4. The method of claim 3, wherein said multi-particulate particles comprise sugar beads.
5. The method of claim 1, wherein said carrier has an enteric coating.
6. A method of treating Inflammatory Bowel Disease comprising administering to a patient a topical formulation of a pharmaceutical composition composed of multi-particulate, enteric-coated particles containing a pharmaceutically effective amount of IL-11.
7. A composition for treating Inflammatory Bowel Disease comprising a topical formulation composed of a pharmaceutically effective amount of IL-11 and a suitable solid carrier.
8. The composition of claim 7, wherein said carrier is an immediate release carrier.
9. The composition of claim 8, wherein said immediate release carrier comprises a collection of multi-particulate particles.
10. The composition of claim 9, wherein said multi-particulate particles comprise sugar beads.
11. The composition of claim 7, wherein said formulation additionally comprises an enteric coating.
12. A composition for treating Inflammatory Bowel Disease comprising a topical formulation composed of multi-particulate, enteric-coated particles containing a pharmaceutically effective amount of IL-11.

# Kaplan Meier Survival Plot Oral IL-11 in Neutropenic Rats

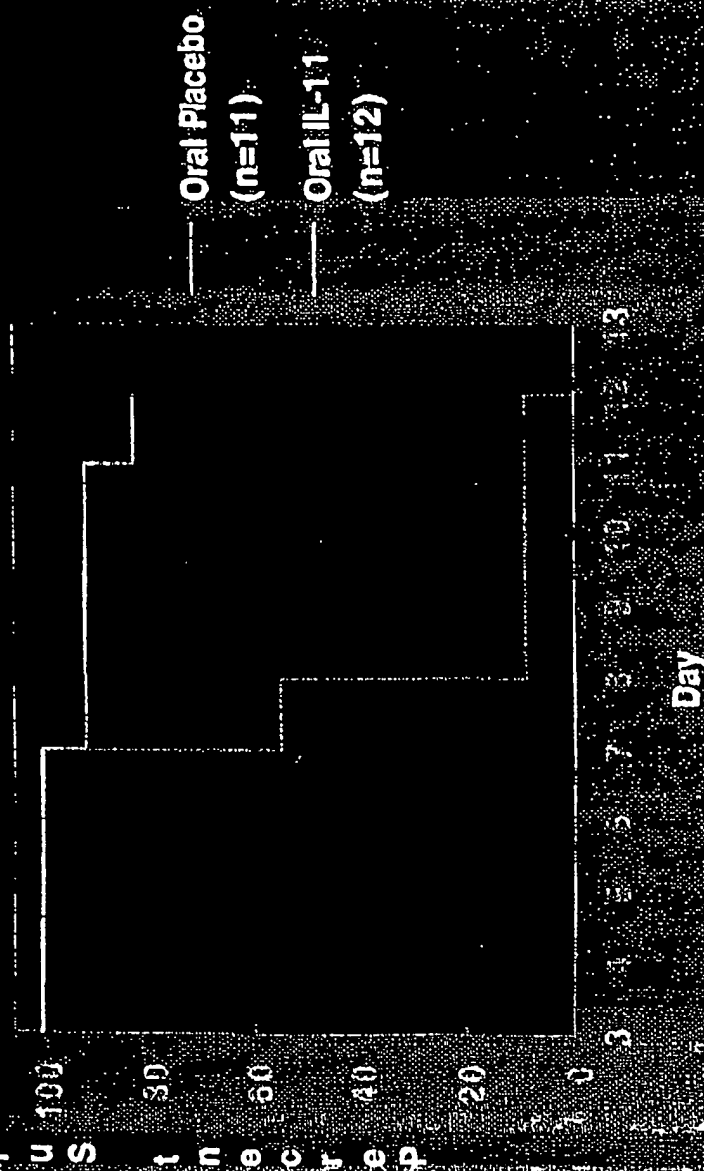


FIG. 1